TERRA, Inc.
Toxicology, Ecology, Research, and Risk Assessment

January 13, 1999

Annie Jarabek
NCEA National Center for Environmental Assessment
3210 Highway 54 Catawba Bldg.
RTP Durham, NC 27709-

RE:
Our Case File: MUSC-6872

Dear Ms. Jarabek:

Dave Mattie asked that I send you a copy of the interim pathology report prepared in relation to the perchlorate research effort ongoing at the Medical University of South Carolina. Although my involvement in the research project has been minimal since submitting the grant proposal, as a consultant I have had to stay informed on the issue. I congratulate you and your colleagues for your success in tackling a complex subject in such a systematic and expeditious fashion. I will forward the pathology analysis of the remaining dose groups to you in the near future. Please feel free to call me with your questions or concerns.

Best Regards,

Alan Warren
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John R. Latendresse, D.V.M., Ph.D.
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Interim Pathology Report
Histopathologic Effects of Ammonium Perchlorate in Thyroid Gland of Mice

Methods

Eight to nine week old male B6C3F1 mice were administered ammonium perchlorate in drinking water for 90 days at 0, 0.1, 1.0, 3.0, and 30 mg/kg/day in two different studies (Studies A and D). For inclusion in this report, only the control and high dose groups from each study were examined. Three histologic sections (A, B, and C) from different levels of the thyroid gland were prepared and submitted for potential histopathologic assessment. Initially, all sections were examined to select the best single section for a detailed evaluation. For consistency in the selection of the region of thyroid gland for the detailed evaluation, only sections of thyroid tissue that contained parathyroid gland were used, when possible. If parathyroid gland was not present, the specimen with the largest area of thyroid gland was used.

Results and Discussion

Morphologies by anatomical site and individual animal are given in the Histopathology Databases (Tables 1 and 2). Thyroid glands from control mice were essentially normal. The follicles were variably sized with complements of relatively large, medium and small colloid-filled lumens. The height of the follicular epithelium was mostly low to medium cuboidal, and the nuclear to cytoplasmic ratio was usually one or less. The cytoplasm of the follicular cells often contained abundant small vacuoles.

The incidence of lesions induced by treatment with ammonium perchlorate is given in the tables 3 and 4. In the 30 mg/kg/day group, although a few peripheral follicles were large with abundant colloid in their lumens, the majority of the follicles tended to be smaller on the average with less colloid compared to controls. Both the inter- and intrafollicular capillaries were mildly congested diffusely, distinguishing them from those of the control thyroid glands. The mildly hypertrophied follicular epithelium was characteristically high cuboidal to low columnar. The nuclear to cytoplasmic ratio of the follicular cells was usually 1.5 to 2. The follicular cells often contained clear perinuclear halos, but the distinct pattern of vacuolization observed in the control group was absent.

Table 3. Study A
Incidence (%) of Thyroid Gland Lesions in Mice Exposed to Ammonium Perchlorate

Anatomical Site	Morphology	Dose (mg/kg/day)	
		0	30
Thyroid follicle	Colloid depletion	0/6 (0)	6/6 (100) [2]*
Capillary	Congestion	0/6 (0)	6/6 (100) [2]
Epithelium, follicular	Hypertrophy	0/6 (0)	6/6 (100) [2]

- [Mean severity]

Table 4. Study D
Incidence (%) of Thyroid Gland Lesions in Mice Exposed to Ammonium Perchlorate

Anatomical Site	Morphology	Dose (mg/kg/day)	
		0	30
Thyroid follicle	Colloid depletion	0/5 (0)	5/5 (100) [2]*
Capillary	Congestion	0/6 (0)	5/5 (100) [1.8]
Epithelium, follicular	Hypertrophy	0/6 (0)	5/5 (100) [1.8]

- [Mean severity]

Inhibition of iodide uptake by the thyroid follicular epithelium has been reported as the mechanism of action of ammonium perchlorate in the rat thyroid gland. Iodination of tyrosine residues of thyroglobulin is one of the essential steps in the production of T3 and T4 (thyroxin). Decreased synthesis of T4 and T3 results in lowered serum concentration that triggers the synthesis and release of TSH from the anterior pituitary gland. TSH receptor activation of cyclic AMP intracellular signaling culminates in hypertrophy of the follicular epithelium. Epithelial hypertrophy, colloid depletion, and the appearance of increased blood flow to the thyroid gland observed in the mice in these studies are consistent with persistent TSH stimulation secondary to deficient production of T3 and/or thyroxin. These observations support a hypothesis of a similar mechanism of action of ammonium perchlorate in the thyroid gland of the mouse that has been shown in the rat.

John R. Latendresse
Diplomate, College of Veterinary Pathologists

Principle Investigator: A. Warren, Ph.D.

Table 1
Ammonium Perchlorate
Histopathology Database

Pathologist: J.R. Latendresse, D.V.M., Ph.D.
Diplomate, ACVP

Study ID	Dose mg/kg/day	Animal ID	Slide ID	Site	Diagnosis	Severity	Remarks
A	0	1	A	thyroid gland	essentially normal tissue		Follicles are variably sized. Follicular epithelium is low to medium cuboidal with the cytoplasmic to nuclear ratio usually equal to or less than 1. Cytoplasm is often vacuolated.
A	0	2	A	thyroid gland	essentially normal tissue		
A	0	4	A	thyroid gland	essentially normal tissue		
A	0	5	A	thyroid gland	essentially normal tissue		
A	0	6	C	thyroid gland	essentially normal tissue		
A	0	3	A	thyroid gland	thyroglossal duct cyst	2	Incidental congenital cyst commonly formed postnatally due to accumulation of proteinaceous fluid in thyroglossal duct remnant.
A	30	28	C	thyroid follicle	colloid depletion	2	
A	30	29	A	thyroid follicle	colloid depletion	2	
A	30	30	A	thyroid follicle	colloid depletion	2	
A	30	25	A	thyroid follicle	colloid depletion	2	
A	30	26	A	thyroid follicle	colloid depletion	2	
A	30	27	A	thyroid follicle	colloid depletion	2	
A	30	25	A	capillary	congestion	2	Inter- and intrafollicular capillaries are prominantly dilated and filled with erythrocytes.
A	30	26	A	capillary	congestion	2	
A	30	27	A	capillary	congestion	2	
A	30	28	C	capillary	congestion	2	
A	30	29	A	capillary	congestion	2	
A	30	30	A	capillary	congestion	2	
A	30	25	A	epithelium, follicular	hypertrophy	2	Follicles are variably sized. Height of the follicular epithelium is usually high cuboidal to low columnar. Area of follicular cytoplasm is usually 1.5 to 2x greater than controls making cytoplamic to nuclear ratio about 1.5 to 2.
A	30	26	A	epithelium, follicular	hypertrophy	2	
A	30	27	A	epithelium, follicular	hypertrophy	3	
A	30	28	C	epithelium, follicular	hypertrophy	2	
A	30	29	A	epithelium, follicular	hypertrophy	2	
A	30	30	A	epithelium, follicular	hypertrophy	2	
A	30	27	A	thyroid gland	thyroglossal duct cyst	2	

Principle Investigator: A. Warren, Ph.D.

Table 2
Ammonium Perchlorate
Histopathology Database

Pathologist: J.R. Latendresse, D.V.M., Ph.D.
Diplomate, ACVP

Study ID	Dose mg/kg/day	Animal ID	Slide ID	Site	Diagnosis	Severity	Remarks
D	0	1	A	thyroid adventicia	ectopic thymus		
D	0	3	A	thyroid gland	essentially normal tissue		Follicles are variably sized. Follicular epithelium is low to medium cuboidal with the cytoplasmic to nuclear ratio usually equal to or less than 1. Cytoplasm is often vacuolated.
D	0	4	A	thyroid gland	essentially normal tissue		
D	0	5	A	thyroid gland	essentially normal tissue		
D	0	6	C	thyroid gland	essentially normal tissue		
D	0	2	A	thyroid gland	thyroglossal duct cyst	2	Incidental congenital cyst commonly formed postnatally due to accumulation of proteinaceous fluid in thyroglossal duct remnant.
D	30	25	C	thyroid follicle	colloid depletion	2	Follicles are predominantly small to medium with decreased luminal size and colloid.
D	30	26	A	thyroid follicle	colloid depletion	2	
D	30	27	A	thyroid follicle	colloid depletion	2	
D	30	28	A	thyroid follicle	colloid depletion	2	
D	30	29	C	thyroid follicle	colloid depletion	2	
D	30	25	C	capillary	congestion	2	Inter- and intrafollicular capillaries are prominantly, diffusely dilated and filled with erythrocytes.
D	30	26	A	capillary	congestion	2	
D	30	27	A	capillary	congestion	2	
D	30	28	A	capillary	congestion	2	
D	30	29	C	capillary	congestion	1	
D	30	25	C	epithelium, follicular	hypertrophy	2	Follicles are variably sized. Height of the follicular epithelium is usually high cuboidal to low columnar. Area of follicular cytoplasm is usually 1.5 to 2x greater than controls making cytoplamic to nuclear ratio about 1.5 to 2. Perinuclear halo often present.
D	30	26	A	epithelium, follicular	hypertrophy	2	
D	30	27	A	epithelium, follicular	hypertrophy	2	
D	30	28	A	epithelium, follicular	hypertrophy	2	
D	30	29	C	epithelium, follicular	hypertrophy	1	
D	30	30	C		NOT EXAMINED		RECUT, NOT ENOUGH TISSUE TO EVALUATE.

From: Latendresse, John <JLatendresse@nctr.fda.gov>
To: 'Alan Warren' <awarren@terra1.com>
Date: Monday, January 11, 1999 3:33 PM
Subject: RE: slides

Alan,
I didn't read your message until after I had sent the report out. With few exceptions, I have never been a strong advocate of "blind" histopathology assessment of toxicology studies. Blind reading generally takes much longer, and it can significantly hinder the identification and characterization of lesions induced by exposure to a xenobiotic agent, particularly when they are suttle. With such a study like ammonium perchlorate, I believe that one would get a much more accurate and confident characterization of morphologic alterations by first comparing the high dose and control specimens to establish thresholds for severity scores, for example. Particularly when lesions are suttle, this is an absolutely essential step precluding one's attempt to determine a dose response. To summarize, frankly, in most instances I believe you don't need a blind reading to get a quality, unbiased assessment by the majority of pathologists who characterize morphologic alterations for a living. Often such requests come from scientists who don't understand the process of morphologic assessment. Most pathologists worth their salt actually do some sort of a blind reading anyway, if the study implies a need. For example, after I have carefully compared the morphology of control and high dose specimens, and detect a putative morphologic alteration believed to be due to exposure to a toxicant, I will confirm my observation by examing a pool of unknown specimens. If I can separate the treatment and control specimens based on the morphologic criteria developed during the high dose and control comparison, I proceed with a similar series of exercises in an effort to define a dose response.